DEVELOPMENT OF SPORANGIUM IN BOTRYCHIUM.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXI.

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(WITH PLATE IX)

THE present work upon Botrychium was taken up with the purpose of investigating what was thought by some to be septation caused by sterilization of mother-cells in the sporangium of *B. ternatum*. Both *B. ternatum* and *B. virginianum* were examined, and the two species were found to be essentially the same in their sporangial development. The material of *B. virginianum* was collected near Woodville, Indiana, March 14, 1904; that of *B. ternatum* was collected near Sullivan, Ohio.

The early stages in the development of the Botrychium sporangium were studied by BOWER (1), CAMPBELL (2), and GOEBEL (5), but apparently none of them studied the interesting later stages. In the present work, though a number of preparations were examined, no special attention was given to the early development further than to confirm the studies of the above mentioned investigators, namely, that the sporogenous mass originates from a single hypodermal archesporial cell. CAMPBELL (2) says that the later divisions in the archesporium do not follow any definite rule, but take place irregularly. This does not accord with my observations, the divisions in *B. ternatum* and *B. virginianum* taking place with great regularity. CAMPBELL implies that his *fig. 129, C*, which shows no regularity in cell-arrangement, is in the mother-cell stage. If this is true, it indicates an unusually small output for a Botrychium. HOLTZMAN (6) made some observations upon the later development of the sporangium of Botrychium, but BOWER (1) thinks that the sequence of segmentations, as shown in HOLTZMAN'S *figs. 3–6*, is not sufficiently intelligible, and should be investigated afresh. He thinks that HOLTZMAN'S description suggests a mode of segmentation more clearly analogous to that in leptosporangiate ferns.

In the sporangium of Lycopodium, the sporogenous mass is divided into blocks of cells, each block apparently being the descendant of a single archesporial cell. The blocking here is evidently related to a multicellular archesporium. In the Botrychium sporangium there occurs a blocking of the sporogenous mass that must have a different cause, as the archesporium is unicellular. The single hypodermal archesporial cell divides usually anticlinally, then periclinally (*fig. 1*). Regular divisions then follow, as shown by *fig. 2*. At the stage shown in *fig. 2* the wall is six to eight cells in thickness, including the tapetum; and the extremely glandular character of the two inner layers of cells indicates that they are definitely set apart as tapetum. In the tapetum at this stage walls are formed both periclinally and anticlinally. The divisions in the sporogenous mass occur with remarkable regularity. The position of the original walls, from stages shown in *figs. 1* and *2*, are still perfectly apparent up to the spore mother-cell stage (*fig. 3*). At this stage the sporogenous mass has somewhat the appearance of the spermatogenous mass in a bryophytic antheridium, except for the size of the cells and the character of the nuclei. By the time the mother-cell stage is reached, the tapetum has become four or five cells thick, is quite glandular in appearance, and there is no further evidence of mitotic division. The sporangium wall-cells adjacent to the tapetum have commenced to collapse as a result of the drain upon them by the tapetum.

Probably the most common and most primitive method of nourishment of mother-cells is by abortion and absorption of a portion of the mother-cells to form a diffuse tapetum, as in Equisetum. Another method is by the formation of sterilized tracts from potentially sporogenous tissue through which material may be conducted to the interior of the sporangium, as the trabeculae of Isoetes or the septations in the microsporangia of *Lemna minor*.

In the sporangium of Botrychium, no sterilization of either kind was found, every mother-cell functioning. So far as I was able to determine, division up to the mother-cell stage is simultaneous throughout the sporogenous mass, yet the original blocks (*fig. 3*) of sporogenous cells still remain perfectly distinct. As the mother-cell enters upon the synapsis stage, the original walls or wall separating

the blocks is apparently thicker, and by the time the spirem is formed the blocks have begun to separate. This separation takes place in the order in which the original walls were laid down in the archesporium and young sporogenous mass (*figs. 3, 4, 6, 7*). At about prophase the mass has separated into at least sixteen (in section) distinct and separated masses (*fig. 6*). "About" prophase is used, since with the separation of the blocks differences in stages of division begin to appear, so that the cells throughout the entire sporogenous mass are not in the same stage, though those in the same block are always in the same stage.

The progressive separation into smaller blocks continues in the same manner in which the first blocks were formed. It takes place along the same lines and in the same order in which the earlier walls were laid down. Whatever stimulus caused the simultaneity of division during the early life of the sporangium seems to have been interfered with here by the cleavage into disjoined blocks, for in the same sporangium the blocks are in different stages of division, it being quite common to find four or five stages. For example, in three adjacent blocks, the cells of one were in early metaphase, of another in telophase, and of the third in anaphase. In another case blocks were found in the same sporangium varying from metaphase of first to metaphase of second division; and it is very common to find them varying from metaphase of first to prophase of second division. One sporangium was found in which all the cells of one-half the entire sporogenous mass were in metaphase, while those of the other half were in telophase. All of the sporogenous tissue throughout its entire development appears in a vigorous and perfectly normal condition.

What may be the cause of this retention of their individuality by the developing sporogenous cells is difficult to say. In regard to bryophytic antheridia this phenomenon has been explained as due to the independent development of the original spermatogenous cells; and there is no further blocking of the spermatogenous mass after the periclinal walls which cut off this mass from the antheridial wall layer have been formed. The difference in rate of development of these blocks has been attributed to differences in food supply, or to some purely physiological cause. It seems that as much might be

said of the blocking in a Botrychium sporangium; though each division, from the very first almost to the formation of the mother-cell, separates masses which retain their individuality throughout their further development. The rate of growth, however, and apparently the food fupply are absolutely the same for all the blocks up to the mother-cell stage. It would seem that in such large sporangia some blocks or cells would be more favorably located than others with reference to food supply or conditions of growth, and there would be greater growth on the part of some regions than others, thus causing irregularities in the arrangement of cells, such as is found in most sporangia, instead of the very regular arrangement in those of Botrychium. Therefore, the above mentioned physiological explanation in regard to bryophytic antheridia is not entirely satisfactory.

What may be the exact cause of this progressive separation of the sporogenous mass along lines where the earlier walls were laid down is impossible at present to say. To me, the most reasonable explanation which can be offered is that the middle lamellae of the walls are acted upon by an enzyme at a time when the sporogenous mass is greatly in need of food. As the lamellae grow older their composition may change, so that they are more easily digested than those more recently formed, thus effecting a progressive separation of the sporogenous tissue. As the blocks become separated, and wholly or partially surrounded by the tapetum, some blocks will of necessity be under slightly different conditions of osmotic pressure or chemical stimulation than others, thus bringing about differences in the rate of their development.

In Botrychium the tapetum is derived from the wall, and absolutely no contribution is made to it from the sporogenous tissue. By the time the sporogenous mass has reached as much as sixty-four cells, the tapetum is clearly delimited from the sporangial wall and is two cells in thickness. From this time on periclinal divisions take place rapidly until the spore mother-cell stage is reached, when the tapetum is four or five cells in thickness and very glandular in appearance. At the first separation of the blocks (*figs.* 3 and 4) in the sporogenous mass—about the synapsis stage—the cell walls of the inner layer of tapetal cells begin to disintegrate. Some of these inner cells are at this time binucleate, while the nuclei of the next one or two layers

of cells are in process of division amitotically, and can be found in all stages. All the tapetal nuclei have now increased much in size, those of the young tapetal cells being 8μ in diameter, and those of the later stage 15 to 20μ. As the blocks of sporogenous cells continue to be formed and more widely separated, the tapetum commences to grow rapidly. The number of nuclei increases greatly, as well as the volume of the cytoplasmic mass in which they float. This pushes inward between the blocks with quite a regular outline (*fig. 6*). At prophase of the first division of the mother-cell, a section of the sporangium shows that these tapetal plates have extended almost across the sporogenous mass between the first formed blocks, and have commenced to grow inward between the blocks of later formation (*fig. 6*). This rapid tapetal growth continues pushing thinner and thinner plates between the smaller blocks as they are formed (*figs. 7, 8, 9*), making a network which finally invests the individual tetrads, or groups of two or four tetrads (*fig. 9*); and at last the spores separate and float in this tapetal mass. The original thicker plates of tapetum may often be found after the spores are completely formed.

As this excessive tapetal growth takes place, the cell walls of the original tapetum break down successively from the inner layers outward, until at metaphase of the mother-cell the walls of only the outermost layer of tapetal cells remain. By the time anaphase of second division (*fig. 8*) is reached, the last walls of the tapetal cells have entirely disappeared, and the inner cells of the sporangial wall begin to take on tapetal characters; especially is this true of the nuclei which resemble tapetal nuclei very closely (*fig. 9*). The cell walls, while they collapse, have not been found to disintegrate entirely, as in the case of the true tapetum. In fact, there is no reason why these inner layers of sporangial wall might not be called tapetum.

Probably the most interesting feature of the development of the Botrychium sporangium is the unusual growth of the tapetum. As before mentioned, it increases greatly in volume and in number of nuclei, yet not a wall is formed anywhere, though it was stained especially for walls. The nuclei are found in all stages of amitotic division (*figs. 5, 8, 9*), but no evidence of mitosis is found. They take stains strongly, are exceedingly large, as mentioned above, and have an unusually thick nuclear membrane. That the nucleus

bears an important relation to the metabolic processes of the cell is too well recognized to need discussion, and this enlargement of nuclei, or increase in nuclear surface, is undoubtedly in response to the increased demand for nourishment on the part of the sporogenous tissue at this stage.

It has been noted frequently that the ovarian follicle cells of arthropods have many large nuclei which divide amitotically, and this has been explained by FLEMMING (4) and CHUN (3) as being a means of securing more rapid metabolic processes between cytoplasm and nucleus through the use of a larger nuclear surface. Our reliable knowledge of the process of amitosis is so meager that one can at this time venture only a tentative explanation of the nuclear behavior in the Botrychium tapetum. Whether amitosis can take place more rapidly than mitosis is unknown, but from the mechanics of the two processes, it would seem that the former would be much the more rapid. WILSON (9) considers that all nuclear division is the response to particular stimuli, and is probably incited by local chemical changes, an idea which is confirmed by PFEFFER (8) and NATHANSOHN (7), who were able to produce mitosis or amitosis at will in *Spirogyra orbicularis*. May we not then look upon amitosis in the tapetum as simply an acquired character due to the unusual demand upon it by the fertile tissue for nourishment at a particular period in its development? Walls being unnecessary, the energy of the organism would not be used in forming them.

As the spores commence to separate in the tetrad, the tapetal cytoplasm has entirely filled the sporangium and many of the nuclei have begun to disorganize, though they seem unusually persistent and many are found after the tetrad is fully formed. Later, when the spores are entirely separated and mature, the tapetum disappears.

Thus we have here worked out the problem of nourishment in a large sporangium by a method entirely different from the two formerly mentioned, namely by the preservation of the individuality of sporogenous cells, thus enabling the mother-cell mass to separate easily into regular blocks, and leaving straight open passageways through which a non-sporogenous tapetum grows rapidly, furnishing the required nourishment for the developing spores. As a general rule there are more spores provided for in a sporangium than nutri-

tive conditions will allow, and there are usually two methods by which this difficulty is overcome: by abortion of mother-cells, and by arrangement for a succession of sporangia according to nutritive supply. In Botrychium, however, there is no abortion of mother-cells and very little difference in the stages of development of the different sporangia in a spike. The nutritive supply is equal to the demand of all mother-cells, probably owing, in part, to the slow growth of the plant, and also to the large amount of food material stored in the stem.

SUMMARY.

1. The sporogenous tissue develops from a single hypodermal archesporial cell.

2. As the successive sporogenous cells are formed, each retains its individuality throughout the development of the sporogenous tissue.

3. Divisions in the sporogenous tissue are simultaneous up to the mother-cell stage.

4. Beginning with the mother-cell stage, the sporogenous mass separates successively into blocks of cells in the same order in which the earlier cells were formed.

5. The blocks develop independently, and at a different rate in the same sporangium, though all cells of one block develop at the same rate. Whatever stimulus caused the simultaneity of division in early sporogenous tissue, is interfered with by separation of the cells into disjoined groups.

6. The progressive separation of thc sporogenous mass is probably caused by the digestion of the middle lamellae.

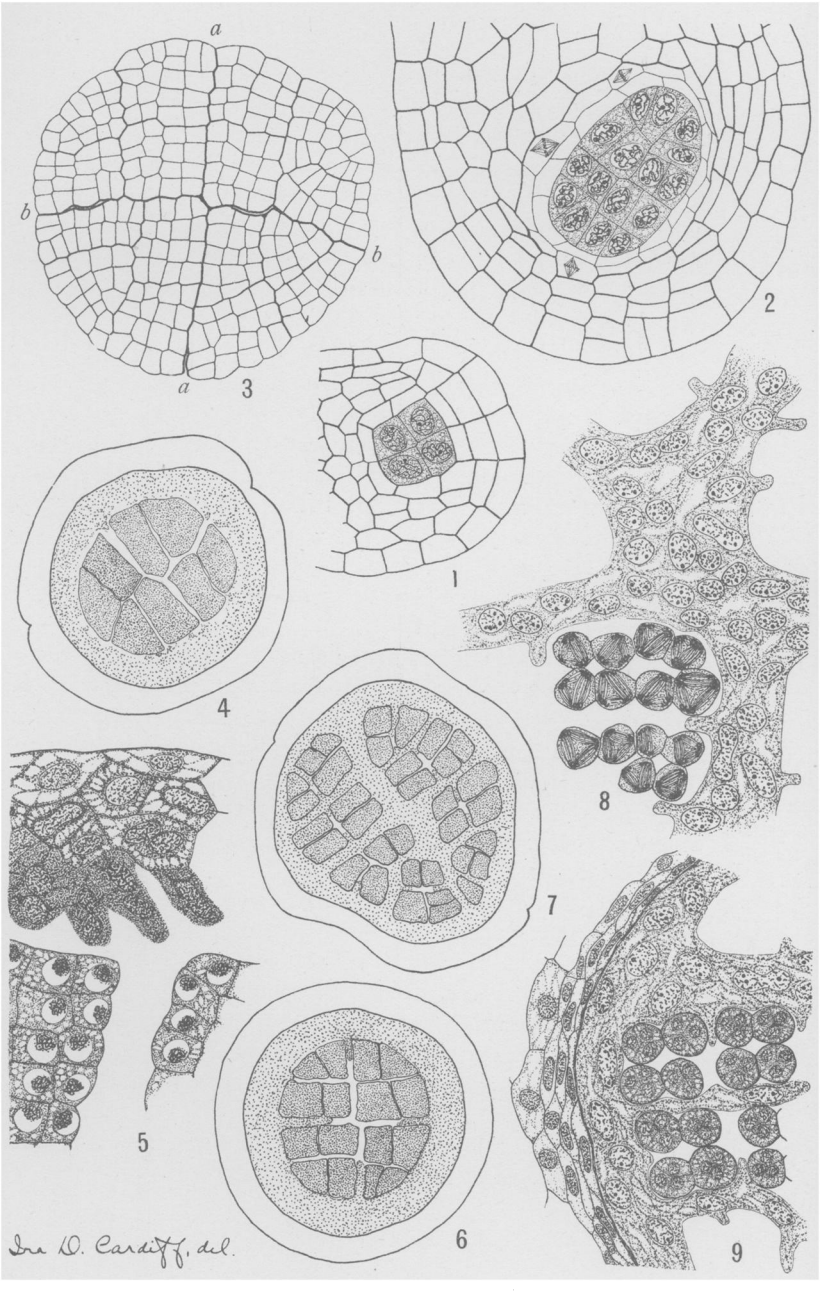
7. All mother-cells produce spores.

8. The tapetum is of non-sporogenous origin.

9. With the separation of mother-cell groups, the tapetum grows rapidly between them without the formation of walls; the nuclei increasing greatly in size, and dividing amitotically.

10. The problem of nourishment in large sporangia may thus be solved by individual development of sporogenous cells, by their later separation into regular groups, and the rapid growth of tapetum between them.

11. The nuclei of the old tapetum are four times the size of those in younger stages of its development.



CARDIFF on BOTRYCHIUM

12. Amitotic division and increase in size of nuclei are both devices for rapidly increasing nuclear surface, thus effecting a larger increase of metabolic products.

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EXPLANATION OF PLATE IX

All figures were made with Zeiss objectives and oculars and Bausch Lomb camera lucida. The original drawings were reduced one-half in reproduction.

FIG. 1. Young sporangium. × 900.

FIG. 2. Sporogenous tissue of thirty-two cells; tapetum two cells thick and divisions taking place both periclinally and anticlinally. × 900.

FIG. 3. Sporogenous tissue in mother-cell stage showing regularity in cell arrangement; the two original walls, *a* and *b*, beginning to separate. × 400.

FIG. 4. Sporangium showing sporogenous tissue separating into blocks along the original walls. × 150.

FIG. 5. Portion of *fig. 4;* mother-cells in synapsis; commencement of tapetal growth. × 900.

FIG. 6. Sporangium showing sporogenous tissue separating along original walls shown in *fig. 2;* plates of tapetum extending between earlier formed blocks. × 150.

FIG. 7. Sporangium showing sporogenous tissue separated into blocks in each of which the cell division is simultaneous. × 150.

FIG. 8. Portion of sporangium showing separation of the forming tetrads, also the large increase in the number of tapetal nuclei. × 900.

FIG. 9. Later stage than shown in *fig. 8*. × 900.